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Antibacterial Properties of Poly(N-Vinylpyrrolidone-co-Acrylic Acid)/ Diethylaminoethanol Ester

P. Hemalatha[1*], M. K. Veeraiah[1], S Prasanna Kumar[2], K.VAnasuya[3], M Manju[4], Raja Naika[5], Lingaraju[5]

[1]Department of Chemistry, Sri Siddhartha Institute of Technology, Tumku, Karnataka, India,
[2]Clov Chem. India Private Limited, Harohalli, Bangalore. Karnataka, India,
[3] Department of Chemistry, Govt. First Grade College and PG Centre, Davanagere, Karnataka, India,
[4]Department of Chemistry, Sri Krishna Institute of Technology, Bangalore, Karnataka, India,
[5]Deprtment of Biotechnology, Tumkur University, Tumkur, Karnataka, India,

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ABSTRACT
Copolymer of N-vinylpyrrolidone (NVP) and Acrylic acid (AA) was prepared by using hydrogen peroxide as initiator in Tetrahydrofuran medium at 45°C under inert atmosphere. Synthesized copolymer was characterized by FTIR, H^1NMR and C^{13}NMR. Copolymer was grafted with N-diethylaminoethanol through Acrylic acid group to form an ester. The antibacterial properties of copolymer and its graft was investigated against four bacterial strains i.e., Gram-ve Klebsiella aerogenes NCIM-2098, Pseudomonas desmolyticum NCIM-2028, and Escherichia coli NCIM-5051 and Gram +ve bacteria like Staphylococcus aureus NCIM-5022. At 150 µg of copolymer dose antibacterial activity was screened in agar media by well diffusion method. All the four bacterial pathogens exhibited significant antibacterial activity in agar well diffusion method when compared with the positive control.

***Key words**: N-vinylpyrrolidone, Acrylic acid, FTIR, H^1NMR, C^{13}NMR, Anti bacterial activity*

1. INTRODUCTION

Recently, there has been a growing concern worldwide about the dangers of microbial contamination in several areas such as medical devices, health care products, water purification systems, hospital and dental equipments etc. Until now progress has been far from sufficience and is evidently lacking the progress of the disease transmission [1, 2]. In general, the conventional disinfectants or antimicrobial agents are solids, liquids or gases of low molecular weight [3]. With the use of these disinfectants or antimicrobial agents, the problems of residual toxicity of the agents which cause more serious problems to the environment cannot be avoided.

Because of the problem associated with the use of conventional antimicrobial agents, the idea of polymeric antimicrobial agents appeared to be attractive alternative. Polymeric antimicrobial agents may enhance the efficacy of some existing antimicrobial agents and minimize the environmental problems accompanying the residual toxicity of the agents in addition to prolonging their lifetime[4-7].

Synthetic polymers with functional groups, which are antimicrobially active are widely used to prevent the growth of microorganisms on the surface of materials such as antifouling paints, antibiotics, in soil sterilization, and in water treatment.

N-vinylpyrrolidone (NVP) has been widely investigated for applications in various fields, [8-12] as these are known to exhibit good biocompatibility due to their hydrophilic nature and low cytotoxicity [13-15]. The amide group of NVP has a high binding affinity for several small and large molecules that are known as good hydrogen-bond acceptors and has been copolymerized with a variety of monomers [16-20]. Homo and copolymers of N-vinylpyrrolidone (VP) are of considerable academic and industrial interest due to their unique properties, allowing the use of these polymer systems in lithography as light sensitive

*Corresponding Author:
E Mail: phlssit@gmail.com

thin coatings for printing plates, for the preparation of separating membranes for ultra filtration, biocompatible polymers with low toxicity and carriers of biologically active compounds, sorbents, coagulants and flocculants [21-25]. Copolymer of 2-hydroxymethylmethacrylate (HEMA) and N-vinylpyrrolidone is used in pharmaceutical applications, such as, cancer therapy [26].

Copolymerization of acrylic acid with NVP helps to modify copolymer by grafting amidation, or cross linking for different applications. Carboxylic acid functional group is one of the functional group that can act as antimicrobial. Cenzig Ozyurek [27] synthesized poly(N-vinylpyrrolidone /Acrylic acid) polyelectrolyte hydrogels by γ ray irradiation and characterized the network structure by swelling measurement. Moshaveria. A [28] synthesized the same copolymer in supercritical fluids and studied the application in dental glass-ionomer cements.

In the previous work, we had synthesized the copolymer of N-vinylpyrrolidone-Acrylic acid copolymer by free radical copolymerization and determined the reactivity ratios [29]. In the present work we synthesized the copolymer N-vinylpyrrolidone - acrylic acid. That copolymer was grafted with N-diethylaminoethanol. The antibacterial activity of the copolymer and the graft against some gram +ve and gram –ve bacteria were screened by Agar well diffusion method [30].

2. EXPERIMENTAL

2.1 Materials

N-Vinylpyrrolidone (Sigma-Aldrich), Acrylic Acid (SD Fine Chemicals) are mixed with Fuller's Earth and kept overnight to settle. Supernatant liquid (washed monomer) is collected separately. Hydrogen peroxide (Spectro Chem) 30% and other solvents are of AR Grade with 99% purity. They are used as received from SD Fine Chemicals, Mumbai, India. N,N-Diethylaminoethanol was purified by distillation.

2.2 Microbial Strains

Antibacterial activity was screened by Agar well diffusion method against four bacterial Strains Gram-ve Klebsiella aerogenes NCIM-2098, Escherichia coli NCIM-5051, Pseudomonas desmolyticum NCIM-2028 and a Gram +ve bacteria Staphylococcus aureus NCIM-5022.

2.3 Synthesis of Copolymer

As both monomers were soluble in water, the copolymerization was carried out in aqueous media. Equi molar concentrations of NVP, AA and water were taken in 100ml three necked round bottom flask with stirrer. Mixture of reaction was heated up to 45^0C, with nitrogen gas flow of 1ml/min, 0.5% (on monomer wt/wt) hydrogen peroxide was added drop wise and heating was continued for 3 hours under nitrogen atmosphere with continuous stirring. After 3hrs, heating was stopped and the mixture of reaction was allowed to cool to room temperature. The high viscous reaction mixture was poured into the Petri dish and evaporated. The copolymer (B) prepared was made to react with N, N-diethylaminoethanol to form an ester through acid group of acrylic acid at 40^0C in DMSO as solvent. The copolymer (B) and its graft (EB) were tested for antibacterial activity.

2.4 Copolymer Characterization

Copolymer is characterized by FTIR spectra using Shimadzu-1800S spectrometer on KBr pellets in

Reaction Scheme

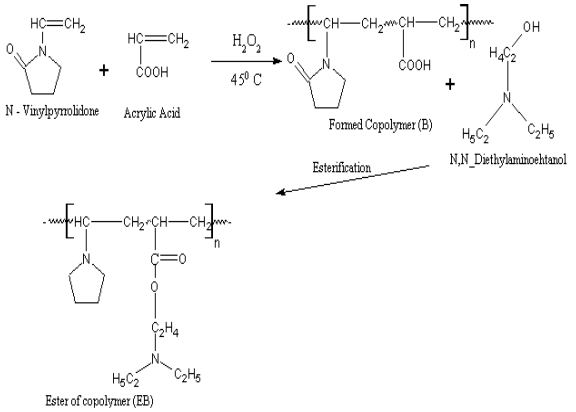

Figure 1: Scheme of preparation of Copolymer and its ester

the range of 400-4000cm^{-1}. The H^1-NMR and C^{13}-NMR (Brucker AMX-400, at Indian Institute of Science, Bangalore) spectra of copolymer is recorded in methanol as solvent and Tetramethylsilane (TMS) as an internal standard.

2.5 Antimicrobial activity test

Antibacterial activity was screened by Agar well diffusion method against four bacterial strains Gram-ve Klebsiella aerogenes NCIM-2098, Pseudomonas desmolyticum NCIM-2028, and Escherichia coli NCIM-5051 and a Gram + ve bacteria Staphylococcus aureus NCIM-5022. Nutrient Agar plates were prepared and swabbed using sterile L-shaped glass rod with 100 µl of 24h mature broth culture of individual bacterial strains. In each petri plate wells were created using sterile cork borer of 6mm.Variedconcentrations of B & EB compounds (1500 µg/well) were used to assess the activity. The compoundsdispersed in sterile water were used as negative control and the standard antibiotic Ciprofloxacin (5µg/50µl) (Hi Media, Mumbai, India) was used as positive control. Then the plates were incubated at 37 °C for

24-36 hours. The zone inhibition was measured in millimeter for every well and noted. Triplicates were maintained in each concentration and the average values were calculated for the ultimate antibacterial activity.

3. RESULTS AND DISCUSSION

3.1 Characterization of Copolymer

FTIR (KBr): 1680, 1724, 1440, 3440, 1330 cm^{-1}.

H^{1-} NMR (400MHz, MeOD): 3.3(4H,1.8, Me), 2.5(H,17.2, Me) 4.2(H,0.93, Me) 1.8(2H, 3.5, Me), 2.8(2H, 2.6, Me), 2.2(2H, 3.5, Me), 11.2(1H, s, COOH).

C^{13-} NMR (400MHz, MeOD): 177 (C), 181 (C), 42 (CH), 30 (CH_2), 35(CH), 68(CH_2), 25(CH_2), 58(CH_2).

3.2 Antibacterial Activity

The antibacterial properties of the given compound (B & EB) were evaluated against Gram-ve K. aerogenes, E. coli, P. desmolyticum and a Gram +ve bacteria S. aureus using Agar well diffusion method. In agar well diffusion method the compounds showed significant antibacterial activity on all the four bacterial strains. The zone of inhibition with the concentration 1500µg per well is as shown in Fig. The data is shown in Table 1.

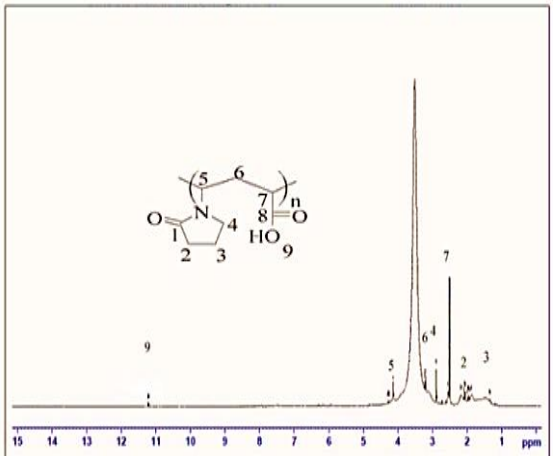

Figure 2: H^{1-} NMR of Copolymer

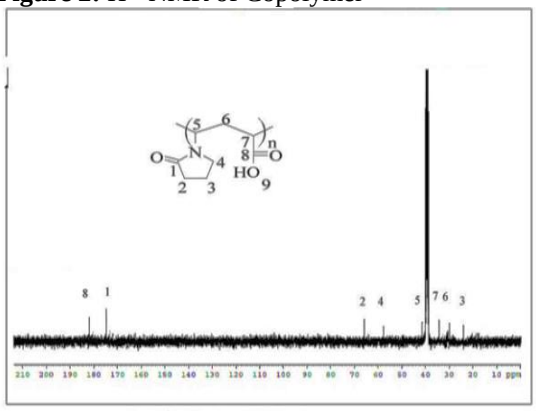

Figure 3: C^{13-} NMR of Copolymer

Table-1: Antibacterial activity of (B & EB) on Pathogenic bacterial Strains

Sl.no	Treatment	Klebsiella aerogenes(Ka) (Mean±SE) (-Ve)	Escherichia Coli (Ec) (Mean±SE) (-Ve)	Staphyloccus aureus (Sa) (Mean±SE) (+Ve)	Pseudomonas desmolyticum(Pd) (Mean±SE) (-Ve)
I	Standard (5µg/50µL)	15.67±0.33**	25.33±0.33**	18.67±0.33**	19.67±0.33**
II	B (1500µg/150µL)	2.67±0.33**	8.00±0.00	5.67±0.33**	4.33±0.33**
III	EB (1500µg/150µL)	2.00±0.00	10.00±0.00	8.33±0.33**	6.00±0.00

Values are the mean ± SE of clear zone in mm. Symbols represent statistical significance, *P < 0.05, **P < 0.01 as compared with the control group.

From this table, it is evident that the copolymer (NVP-AA) and its grafted ester are showing a better inhibition for all bacteria. In all the bacteria, Ester of the copolymer is showing more activity as it has amine group in it, which is more antibacterial than carboxylic acid group.

4. CONCLUSION

The Copolymer (NVP–AA) was synthesized. Its structure was confirmed by FTIR, H^1 NMR and C^{13} NMR. It was made to react with DEEA for esterification. The anti bacterial activity of copolymer and its graft was determined by agar

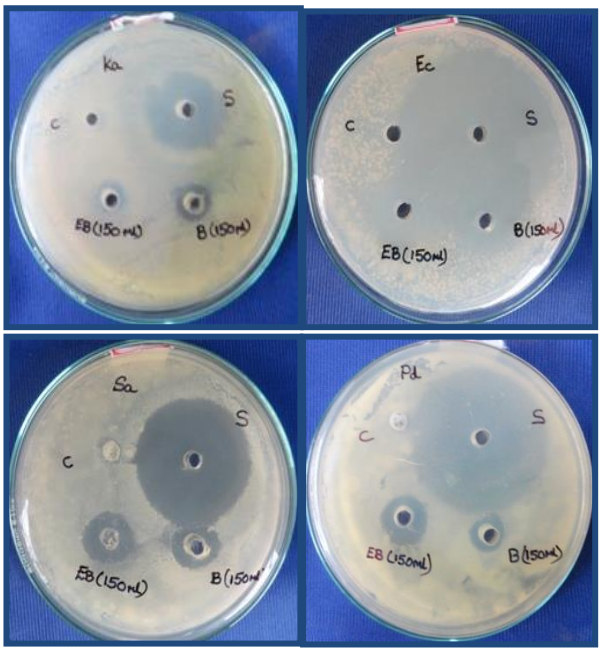

Figure 4: The pictures show Agar well diffusion (zone of inhibition) results of (Ka) Klebsiella aerogenes (Ec) Escheriachia coli (Sa) Staphylococcus aureus and (Pd) Pseudomonas desmolyticum.

well diffusion method. It was demonstrated that the copolymer and its graft have a positive effect in controlling the growth of the gram -ve and gram +ve bacteria. This new polymeric biocide system can be recommended for the biomedical and food industry application.

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